

Syn-PER™ Synaptic Protein Extraction Reagent

87793

2382.0

Number**Description**

87793

Syn-PER Synaptic Protein Extraction Reagent, 100mL, sufficient reagent to extract synaptic protein from ~10g of neuronal tissue and primary cultured neurons

Storage: Upon receipt store product at 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific Syn-PER Synaptic Protein Extraction Reagent extracts proteins expressed in the synapses of neuronal tissue and primary cultured neurons. Native pre- and post-synaptic proteins extracted by Syn-PER Reagent are suitable for downstream applications, including Western blots, enzymatic activity assays (e.g., phosphatase and kinase), protein-protein interaction studies and immunoprecipitations. Synaptosomes from prepared neuronal tissue can be used to study neurotransmitter release. Additionally, Syn-PER Reagent minimizes phosphoprotein degradation and does not require ultracentrifugation.

Important Product Information

- Syn-PER Reagent does not contain protease or phosphatase inhibitors. Add necessary inhibitors to Syn-PER Reagent immediately before use to a final inhibitor concentration of 1X (see Related Thermo Scientific Products).
- Syn-PER Reagent does not contain antimicrobial agents. Avoid contaminating the opened bottle.
- Perform all steps, including homogenization and centrifugation, at 4°C to reduce proteolysis, dephosphorylation and denaturation. Store samples and extracts on ice.

Additional Materials Required

- Ice-cold phosphate-buffered saline (PBS, Product No. 28372): 0.1M sodium phosphate, 0.15M sodium chloride; pH 7.2
- Refrigerated bench-top microcentrifuge
- Dounce tissue grinder
- EDTA-free protease, phosphatase or combination inhibitors (see the Thermo Scientific Related Products Section)

Procedure for Synaptic Protein Extraction from Neuronal Tissue

- Place the Dounce tissue grinder on ice before use.
 - Immediately before use, add inhibitors to the Syn-PER Reagent; add inhibitors only to the amount being used for the procedure and not to the stock solutions.
1. Weigh neuronal tissue samples. Add 10mL of Syn-PER Reagent per gram of tissue (e.g., 2mL of Syn-PER Reagent per 200mg of brain tissue).
 2. Perform Dounce homogenization on ice with ~10 slow strokes.
 3. Transfer homogenate to an appropriate centrifuge tube(s).
 4. Centrifuge the tube at 1200 × g for 10 minutes at 4°C. Discard the pellet and transfer supernatant to a new tube. If required, save a sample of the supernatant (homogenate) for analysis.
 5. Centrifuge supernatant at 15,000 × g for 20 minutes at 4°C.

6. Remove the supernatant from the synaptosome pellet. If required, save the supernatant (cytosolic fraction) for analysis.
7. Add 1-2mL of Syn-PER Reagent per gram of sample to suspend the synaptosome pellet (e.g., 500 μ L for 200-400mg of brain tissue).

Note: Recommended volumes should result in 3-4 μ g/ μ L of synaptic protein.

8. Maintain the synaptosome suspension on ice until performing neurotransmitter release studies or downstream applications.

Note: The synaptosome suspension can be stored in 5% (v/v) DMSO at -80°C or in liquid nitrogen for extended periods of time; however, a substantial reduction in synaptosome viability occurs after prolonged storage. For best results, perform activity studies (e.g., calcium-dependent neurotransmitter release) with new synaptosomes.

Note: Further synaptosome suspension separation into pre- and post-synaptic protein fractions and synaptic vesicle proteins is done by applying the synaptosome suspension onto a discontinuous sucrose gradient followed by prolonged ultracentrifugation.

Procedure for Synaptic Protein Extraction from Primary Cultured Neurons

1. Carefully decant culture medium from cells. Wash cells twice with ice-cold PBS.
2. Add the appropriate amount of Syn-PER Reagent to the plate or each plate well (see Table 1).

Table 1. Suggested volume of Thermo Scientific Syn-PER Reagent to use for different sizes of standard culture plates.

<u>Plate size/Surface area</u>	<u>Syn-PER Reagent volume</u>
100mm	500-1000 μ L
60mm	250-500 μ L
35mm*	200-400 μ L

*Primary cultured neurons grown for 3 weeks in a 35mm plate containing 10⁶ cells yield ~4.0 μ g of synaptic protein.

3. Scrape the plate surface using a cell scraper to lift the cells. Collect the lysate and transfer to a microcentrifuge tube.
4. Centrifuge sample at 1200 \times g for 10 minutes at 4°C. Discard the pellet and transfer supernatant to a new tube. If required, save a sample of the supernatant (homogenate) for analysis.
5. Centrifuge supernatant at 15,000 \times g for 20 minutes at 4°C.
6. Remove the supernatant from the synaptosome pellet. If required, save the supernatant (cytosolic fraction) for analysis.
7. Suspend the synaptosome pellet in Syn-PER Reagent; final volume is dependent on the size of the culture dish (e.g., 20-40 μ L per sample for a 35mm dish).
8. Maintain the synaptosome suspension on ice until performing neurotransmitter release studies or downstream applications.

Note: The synaptosome suspension can be stored in 5% (v/v) DMSO at -80°C or in liquid nitrogen for extended periods of time; however, a substantial reduction in synaptosome viability occurs after prolonged storage. For best results, perform activity studies (e.g., calcium-dependent neurotransmitter release) with new synaptosomes.

Note: Further synaptosome suspension separation into pre- and post-synaptic protein fractions and synaptic vesicle proteins is done by applying the synaptosome suspension onto a discontinuous sucrose gradient followed by prolonged ultracentrifugation.

Related Thermo Scientific Products

78420	Halt™ Phosphatase Inhibitor Cocktail (100X), 1mL
87785	Halt Protease Inhibitor Cocktail, EDTA-free (100X), 1mL
87786	Halt Protease Inhibitor Cocktail Kit, 1mL
78440	Halt Protease and Phosphatase Inhibitor Cocktail (100X), 1mL
78441	Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X), 1mL
88660	Pierce® Protease Inhibitor Tablets, 30 tablets
88661	Pierce Protease Inhibitor Tablets, EDTA-free, 30 tablets
88662	Pierce Phosphatase Inhibitor Tablets, 20 tablets
88663	Pierce Protease and Phosphatase Inhibitor Tablets, 20 tablets
88664	Pierce Protease and Phosphatase Inhibitor Tablets, EDTA-free, 20 tablets
87791	Pierce Tissue Strainers, 250µm, 50 each
87792	N-PER® Neuronal Protein Extraction Reagent, 100mL
87790	Subcellular Protein Fractionation Kit for Tissues
23225	Pierce BCA Protein Assay Kit
15041	Pierce 96-Well Plates, Corner-notch, 100/pkg
28372	BupH™ Phosphate Buffered Saline Packs, 40 packs
28348	20X Phosphate Buffered Saline, 500mL

General References

Bai, F., *et al.* (2007). Synaptosome proteomics. *Subcell Biochem* **43**:77-98.

Baldwin, M.L., *et al.* (2003). Two modes of exocytosis from synaptosomes are differentially regulated by protein phosphatase types 2A and 2B. *J of Neurochem* **85**:1190-9.

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